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Patient With an Xp21 Contiguous Gene Deletion Syndrome in Association With Agenesis of the Corpus Callosum

Sergio E. Baranzini,[1,2]* Graciela del Rey,[3] Nora Nigro,[4] Irene Szijan,[1,2] Néstor Chamoles,[5] and Juan C. Cresto[4]

[1]Programa de Neurobiología Molecular, Hospital de Clínicas "José de San Martín", Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
[2]Cátedra de Genética y Biología Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina
[3]Centro de Investigaciones Endocrinológicas (CEDIE-CONICET), Hospital de Niños R. Gutiérrez, Buenos Aires, Argentina
[4]Servicio de Endocrinología, Hospital Pedro de Elizalde, Buenos Aires, Argentina
[5]Fundación para el Estudio de las Enfermedades Neurometabólicas, Buenos Aires, Argentina

The so-called Xp21 contiguous deletion syndrome or complex glycerol kinase deficiency (GKD) usually presents with classical Duchenne muscular dystrophy (DMD) or a milder dystrophic myopathy, adrenal hypoplasia, and GKD. A number of syndromic and nonsyndromic cases of agenesis of the corpus callosum (ACC) also map to that location. To date, none of the cases of complex GKD have been associated with ACC. Here, we report on a patient with a complex phenotype as a result of the Xp21 contiguous deletion syndrome in association with ACC. Biochemical, cytogenetic, and molecular analyses were performed to detect and establish the size of the genomic deletion. It is at least 3 million base pairs in length; however, exact limits could not be determined in the present study. Nevertheless, we suggest the presence of a primary gene involved in the embryogenesis of the corpus callosum between Xp21.1 and Xp22.11. Am. J. Med. Genet. 70:216–221, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: contiguous deletion syndromes; complex GKD; X-linked; agenesis of the corpus callosum

INTRODUCTION

The association of Duchenne muscular dystrophy (DMD), glycerol kinase deficiency (GKD), and congenital adrenal hypoplasia (AHC) is referred to as a contiguous gene deletion syndrome, which involves the short arm of X chromosome [Cohen and Cole, 1989; Greenberg, 1993]. Patients with this complex phenotype may have classical DMD or a milder dystrophic myopathy, as seen in the 2 brothers first described with this disorder [McCabe et al., 1977]. GKD is characterized by glyceroluria, hyperglycerolemia, psychomotor retardation, spasticity, growth failure, myopathy, osteoporosis, and adrenal insufficiency. Routine clinical laboratory tests may play an important part in identifying these associations and may significantly influence the patient's quality of life.

Although these patients are also mentally retarded, no structural central nervous system abnormalities have been reported to date. The patient reported here not only shares the typical signs and symptoms of those with the Xp21 contiguous gene deletion syndrome, but also has agenesis of the corpus callosum (ACC).

MATERIALS AND METHODS

Patient-Case Report

A male infant was delivered at term without significant perinatal complications; the mother was 42 years old. She was an alcoholic and it was her fifth pregnancy. Previously she had had 2 apparently normal girls and 2 boys. The first child died during delivery while the second resulted in spontaneous death close to the date of birth.

At 12 days, the infant developed severe dehydration, hyponatremia (sodium, 117 mEq/L), and hyperka-

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I.S. and J.C.C. are established investigators of CONICET.

*Correspondence to: Sergio Baranzini, Biochem., Cátedra de Genética y Biología Molecular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113, Capital Federal, Argentina.

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lemia (potassium, 8.8 mEq/L). Subsequent analysis showed normal plasma 17α-hydroxyprogesterone (0.73 ng/ml) and increased renin plasma activity (42 ng/ml/h). Fludrocortisone treatment (at 0.05 μg/kg) corrected the electrolyte abnormalities and the patient improved rapidly. He was examined periodically for management of his adrenal insufficiency. Over the next few months he showed signs of developmental delay and growth failure. A computed tomography scan (CT) showed ACC with a subtle ventricular dilatation (Fig. 1).

At age 7 years, he had muscle pain and fatigue. Serum total creatine kinase (CK) activity was 2,160 U/L and skeletal muscle findings were histopathologically consistent with DMD. Urinary and serum glycerol levels were increased.

The latest studies (10 years) showed serum CK activity of 5,700 U/L, low morning cortisol (<1 μg%), plasma 17α-hydroxyprogesterone of 0.1 ng/ml (normal value is less than 2 ng/ml), adrenocorticotropic hormone (ACTH) of 72 pg/ml (normal value is less than 57 pg/ml), and aldosterone of 15 pg/ml (normal range: 35 to >350 pg/ml). The patient has a hyperteloric appearance, with alternating strabismus, wideset eyes, and a drooping mouth (Fig. 2a). Ophthalmoscopic findings were normal. Facial measurements were as follows: outer and inner canthal distance were 11.5 and 3.8 cm, respectively, interpupillary distance was 5.5 cm, philtrum was 1.8 cm, and total ear length was 5.4 cm. His hands showed clinodactyly and no abnormality was seen in his palms (Fig. 2b). At this point the diagnosis of contiguous gene deletion syndrome was postulated and additional studies were undertaken to confirm the diagnosis.

Organic Acids Analysis

Organic acids were extracted with ethyl acetate from acidified urine. The extract was evaporated and the residue derivatized by trimethyl silylation reagent (BSTFA/1% TMCS) according to Duran et al. [1980]. The organic acids were separated by capillary gas chromatography on a GC Hewlett-Packard 5890 equipped with a 25 m (0.25 mm/id) WCOT fused silica column, CP SIL 19-cb (CHROMPACK, The Netherlands). Peak identities were established by mass spectrometry with an HP 5971 A mass selective detector (Hewlett-Packard, USA) by comparing mass spectra to those of authentic compounds. Glycerol was determined enzymatically/spectrophotometrically using the conversion of glycerol to glycerol-1-P by glycerolkinase [Eggstein and Kuhlmann, 1970].

Cytogenetic Analysis

Cytogenetic analyses were performed using standard cell culture techniques [Moorhead et al., 1960] and a high-resolution cell culture technique for prometaphase chromosomes. Prometaphase chromosome preparations for high-resolution analyses were performed by a modification of the technique of Yunis [1976]. G-banded patterns of elongated chromosomes were obtained by 5-fluodeoxyuridine (FrdU) and 5-bromodeoxyuridine (BrdU). Banding patterns obtained here were compared with previously published standard banding patterns [ISCN, 1985]. At least 100 cells were scored in both cultures and all metaphases from all cultures were photographed as well.

Southern Blotting

High molecular weight genomic DNA was isolated from leukocytes as described elsewhere [Maniatis et al., 1982]. Genomic DNA was digested with Hind III, Eco RI, and Taq I (Bethesda Research Laboratories, Gaithersburg, MD). Seven microgram aliquots of digested DNA were electrophoresed on a horizontal 1% agarose (Sigma Chemical Co., St. Louis, MO) gel for 16 hours at 50 V. DNA was transferred to a nylon membrane (Zetaprobe, BioRad Laboratories, Richmond,

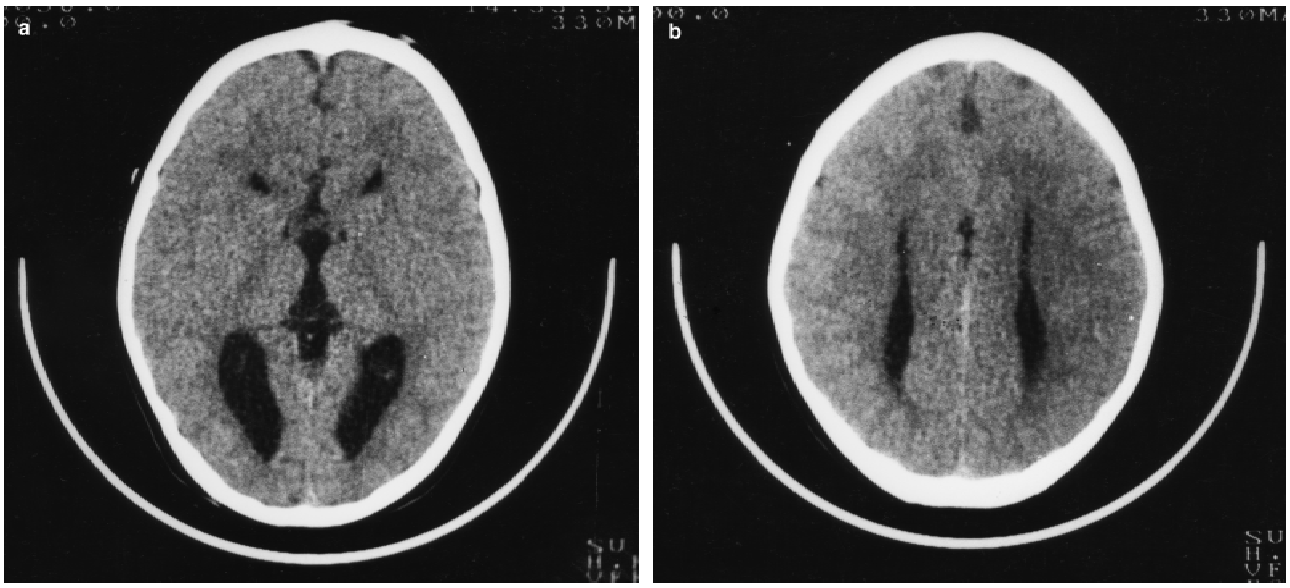

Fig. 1. CT scans showing ACC. **a:** Axial slice of a CT. A cystic dilation dorsal to the third ventricle as well as a subtle dilation of lateral ventricles can be seen. **b:** Axial slice of a CT on an upper plane. Lateral ventricles adopt a parallel conformation.

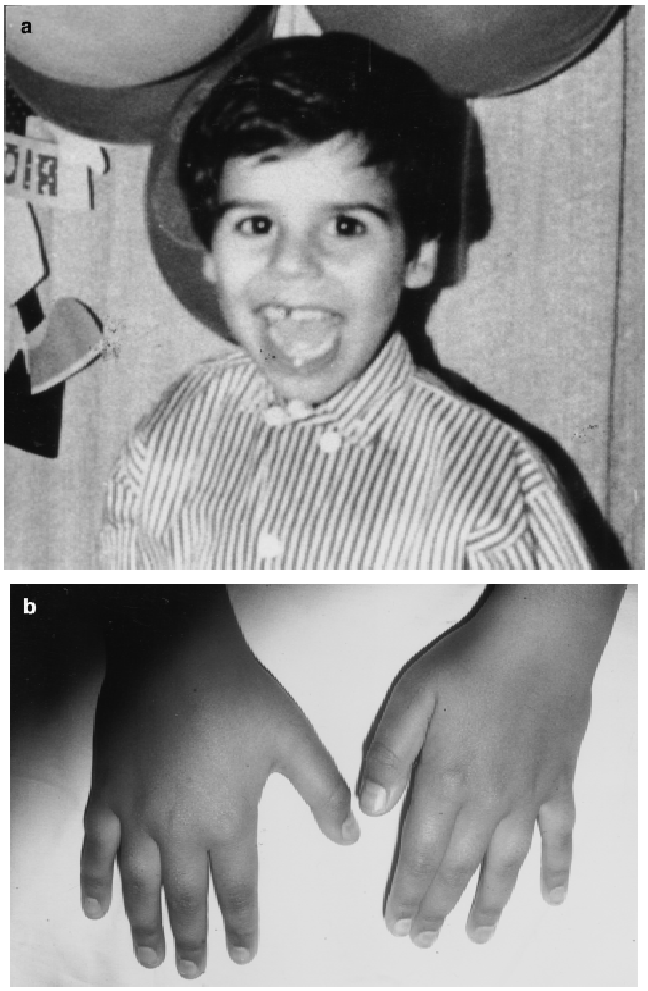

Fig. 2. **a:** Dysmorphic features on patient's face. Hypertelorism, alternating strabismus, and drooping mouth are shown. Forehead and eyebrows as well as the root and bridge of the nose are also characteristic. **b:** Patient's hands.

CA) according to the method of Southern [1975] adapted to use 0.4 N NaOH as the transfer solution [Reed and Mann, 1985]. Blots were prehybridized at 42°C in a 50% formamide buffer containing 7% sodium dodecyl sulfate (SDS) and 0.25 M PO_4HNa_2. Probes were labeled to high specific activity (10^8 dpm/μg) by using random primers (Megaprime™, Amersham) [Feinberg and Vogelstein, 1983]. Hybridization was performed for 18 hours at 42°C in the same buffer as prehybridization. Blots were exposed to radiographic films (Kodak X-OMAT, Rochester, NY) for 1–9 days.

Probes

The human insert of plasmid pDP1007 is a 1.3 Kb Hind III fragment purified from phage lambda ϕX82 which codes for a part of the ZFX and ZFY genes [Schneider-Gädicke et al., 1989]. This insert was used to map the telomeric end of the deletion. Dystrophin cDNA probes 1–2a, 2b–3, 4–5a, 5b–7, 8, and 9–14 have been described by Koenig et al. [1987]. Instead of the whole cDNA 9–14, a Bam HI fragment containing the cDNA10 region was used to confirm the centromeric deletion breakpoint.

Polymerase Chain Reaction (PCR)

We used genomic primers (sequence available upon request) to randomly amplify several exons of the DMD gene in order to establish the centromeric breakpoint. Amplifications were performed in 25 μl volumes containing 125 ng genomic DNA; 0.5 μmol/L each primer; 1X Taq Polymerase buffer (67 mmol/L Tris-HCl, pH 8.8, 16.6 mmol/L $(NH_4)_2SO_4$, 6.7 mmol/L $MgCl_2$, 170 μg/ml BSA, 10 mmol/L 2-mercaptoethanol); 0.2 mmol dNTPs; and 1.0 unit of Taq DNA Polymerase (Amplitaq, Cetus). Cycling conditions were 94°C for 3 minutes, followed by 40 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds with a final extension of 72°C for 5 minutes. The products of amplification were visualized directly by electrophoresis of 10 μl of product in horizontal 2% agarose minigels after staining with ethidium bromide at 0.5 μg/ml.

RESULTS

Urinary Organic Acids

Due to the increased urinary glycerol eliminated, a prominent peak corresponding to the trimethylsilyl ester of glycerol was the only abnormality observed. The corresponding values were urinary 116 mmol/mmol creatinine (normal is less than 0.05 mmol/mmol creatinine) and serum 2.1 mmol/L (normal is less than 0.25 mmol/L).

Cytogenetic Analysis

G-banded chromosomes in standard preparations appeared normal. At low resolution (300–400 bands), the distance between bands Xp21 to Xp22 appeared thinner in the patient's X chromosome. High resolution (700 bands) of prometaphase chromosomes showed a deleted X chromosome in the patient when compared to a normal control. Band Xp21 in the patient is about half its normal size, and is involved in a subtle interstitial deletion.

Cytogenetically, this deletion comprises part of bands Xp21.1 and Xp21. Thus, the corresponding karyotype is 46,del(X)(p21.1–p21.3),Y.

Molecular Analysis

The patient's DNA was first analyzed by PCR amplification using some of our 56 genomic primers for the DMD gene in order to establish the centromeric startpoint of the deletion. The gels showed appropriately sized amplicons up to and including exon 60, but no product was detected from the amplicon within exon 61 onward up to exon 79 (data not shown), the last exon in the dystrophin gene.

Southern blot analysis of genomic DNA with probe cDMD10 gave a hybridization signal only for the 3.5 kb Hind III fragment (data not shown) which corresponds to exon 60 but showed no signal for those fragments of 6.6, 2.8, 12.0, 2.4, and 2.5 kb which correspond to the next 5 exons, respectively.

In order to locate the telomeric deletion breakpoint,

we first analyzed the amelogenin locus (Amelx) by PCR amplification. This gene was found to map to Xp22.31 by Nakahori et al. [1991]. Normal-sized fragments were obtained when compared to controls as shown in Figure 3.

Hybridization of Southern blots of either Eco RI or Taq I digests of genomic DNA with probe pDP1007 (ZFX) gave signals of expected sizes when compared to controls (Fig. 4).

DISCUSSION

The terms "Xp21 contiguous gene syndrome" or "complex GKD" have been used to describe patients with multiple single-gene disorders originating from deletion or duplication of an Xp21 segment. Typically, they have loss of the GKD locus together with one or more closely flanking genes, most frequently AHC and DMD. Contiguous gene deletion syndromes are characterized by the fact that they are usually sporadic. Although the cytogenetic abnormality may be detectable by high resolution karyotyping, some may be so small as to be undetectable either by this method or by fluorescence in situ hybridization (FISH). The clinical manifestations in patients with complex GKD can be nonspecific; however, life-threatening adrenal insufficiency may develop as a result of deletion of the AHC gene. Recognition of the distinct developmental anomalies associated with complex GKD is one way of facilitating the clinical diagnosis of this syndrome. Several patients with the syndrome have shown failure to thrive, psychomotor retardation, and short stature or abnormal genitalia.

Neurological abnormalities include spasticity with weakness and a particular "handwashing" motion that resembles that of those with Rett syndrome [Scheuerle et al., 1995]. None of the Xp21 contiguous gene deletion syndromes described to date mention corpus callosum abnormalities as a characteristic. Corpus callosum abnormalities, in fact, have been described in a number of nonrelated conditions [Vles et al., 1993; DeMarco, 1992; Schaefer et al., 1991; Kolodny, 1989] including some X-linked syndromes. Thus, they seem to be a multigenic trait in which several autosomal and X-linked loci take part. Aicardi syndrome [Gorrono-Echebarria, 1993] is one of the X-linked conditions that include ACC. Our patient has complex GKD and partial ACC, but clearly does not have the Aicardi or FG syndrome.

By using high-resolution cytogenetic analysis and molecular techniques, we found the genomic abnormality in our patient to be an Xp21 deletion. The centromeric startpoint of the deletion was localized to the 3′

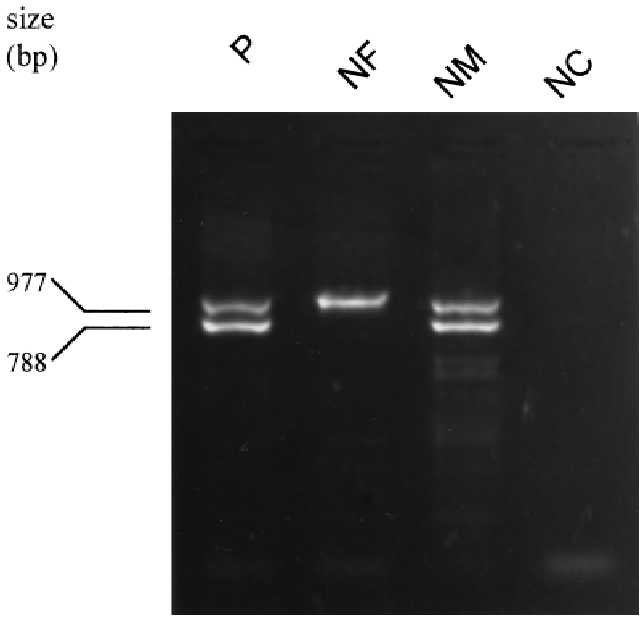

Fig. 3. Agarose gel electrophoresis of Amelx PCR products. Specific amplification products from the patient (P), a normal female control (NF), and a normal male control (NM). The negative control (NC) reaction tube contains everything except DNA. The length of each fragment is measured in base pairs (bp).

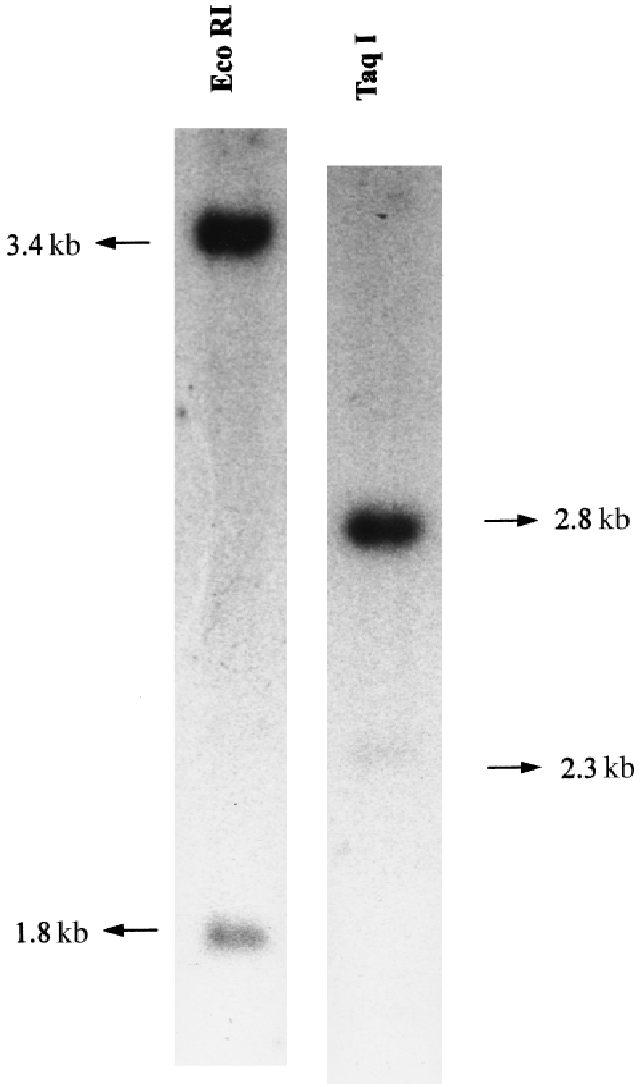

Fig. 4. Autoradiograms for Southern transfers of genomic DNA hybridized to probe pDP1007. Patient's genomic DNA digested either with Eco RI or Taq I was blotted onto nylon membranes and hybridized to ^{32}P-labeled probe pDP1007. Arrows indicate hybridization signals of expected sizes, demonstrating the presence of the respective genomic sequences (see Fig. 5).

end of the DMD gene, between exons 60 and 61. The fact that only the 3.5 kb Hind III fragment (which corresponds to exon 60) showed a positive signal when hybridized to cDNA10 probe gave us the basis to establish the deletion startpoint. In addition, PCR amplification of DMD exon 60 yielded a normally sized amplicon while no amplification products were obtained when analyzing exons 61 up to 79, which is the last exon in the dystrophin gene.

The telomeric end of the deletion was first analyzed by partial amplification of the amelogenin locus. The normally sized amplicons obtained showed their presence, thus indicating that the second deletion breakpoint might be towards the centromere. In addition, the expected hybridization signals demonstrated by probe pDP1007, both with Eco RI and Taq I in the patient's genomic DNA, indicated the presence of the ZFX locus.

Altogether these data suggest that the deletion starts next to DMD exon 60, spans at least 3 million base pairs, and might extend to somewhere between the AHC (Xp21.3) and ZFX loci (Xp22.11) (Fig. 5). According to the AGIS (Agricultural Genome Information Server), Human Chromosome X Database, the latter seems to be approximately 5 cM apart.

Proud et al. [1992] suggested that a primary gene involved in the embryogenesis of the corpus callosum may be located between Xp11.3 and Xp21.3 on the basis of linkage analysis with a number of X-linked probes. Nevertheless, we can demonstrate the presence of the region between Xp11.3 and Xp21.1 (to which DMD gene maps) since our patient's deletion starts just next to DMD gene exon 61 and no evidence exists for another deletion towards the centromere. In this context, our data suggest that the Xp deletion responsible for complex GKD and ACC might span, at most, the region between Xp21.1 and Xp22.11, thus narrowing the region to which a gene involved in ACC might map.

To our knowledge, there are a number of genes that have been linked to this region. A locus for nonsyndromic X-linked sensorineural hearing impairment (deafness 4, congenital sensorineural) linked to Xp21.2 was suggested by Lalwani et al. [1994]. In addition, a couple of loci for RPXL (RP-2 and RP-3) have been mapped by linkage analysis to the short arm of the X chromosome [Bhattacharya et al., 1984; Nussbaum et al., 1985], but are centromeric to the deletion described in this report. A new locus for retinitis pigmentosa X-linked (RPXL) showing linkage to DXS28 (RP-6) cannot be excluded to date [Musarella et al., 1988; Ott et al., 1990].

In this connection, no evidence of either hearing impairment or retinal pathology was observed in our patient. Further analysis with either the B24 (DXS67) or C7 (DXS28) probes will help in determining the extension of the deletion.

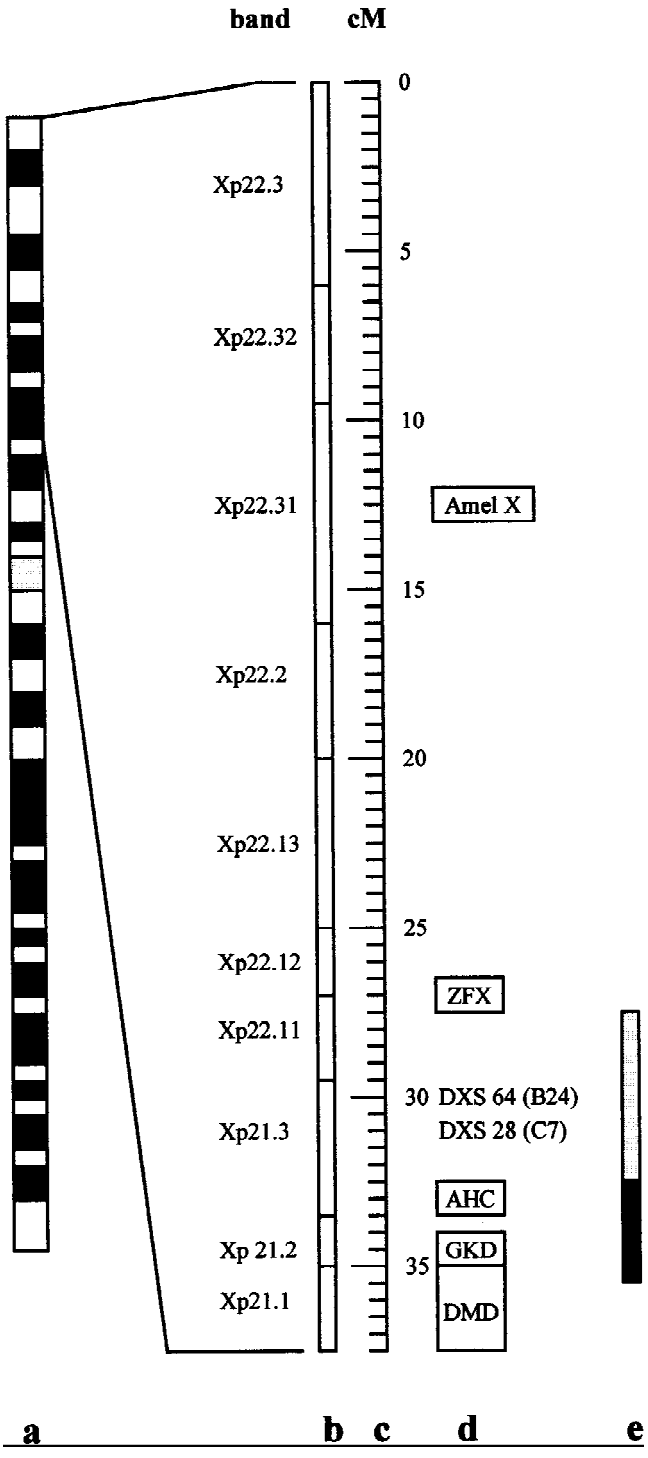

Fig. 5. Genetic and physical map showing the relative positions of the loci analyzed and approximated genetic distances. **a:** An idiogram of the X chromosome. **b:** An expanded view of the Xp21.1-Xpter region is depicted. **c:** The genetic distance is given in centiMorgans. **d:** Boxed names are the loci analyzed or known to be deleted. Other names are well-characterized loci and their respectives probes in brackets. **e:** The solid bar represents the minimal deleted region. The telomeric breakpoint interval is represented by the dotted bar.

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